Protective influences of α-ketoglutarate on lipid peroxidation and antioxidant status in ammonium acetate treated rats

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The effects of α-ketoglutarate on ammonium acetate induced hyperammonemia were studied biochemically in experimental rats. The levels of circulatory, non-protein nitrogen, serum transaminases and thiobarbituric acid reactive substances were significantly increased in ammonium acetate treated rats. These levels were significantly decreased in α-ketoglutarate and ammonium acetate treated rats. Similar patterns of alterations were observed in the levels of free fatty acids, triglycerides, phopholipids and cholesterol in between various groups. Further non-enzymatic (vitamins C and E) and enzymatic (superoxide dismutase and catalase) antioxidants were significantly decreased in ammonium acetate treated rats; and were significantly increased in α-ketoglutarate and ammonium acetate treated rats. The biochemical alterations during α-ketoglutarate treatment could be due to (i) the detoxification of excess ammonia, (ii) by participating in the non-enzymatic oxidative decarboxylation in the hydrogen peroxide decomposition process and (iii) by enhancing the proper metabolism of fats which could suppress oxygen radicals generation and thus prevent the lipid peroxidative damages in rats.

Ammonia is formed in mammals and humans as a product of catabolism of proteins and other nitrogenous compounds. At high levels, ammonia is neurotoxic, leading to functional disturbances of the central nervous system, which can lead to coma and death. Ammonia intoxication impairs mitochondrial function which could lead to decreased ATP synthesis and also to increased formation of free radicals. The major toxic effects of ammonia probably involve changes in cellular pH and the depletion of certain citric acid cycle intermediates, particularly α-ketoglutarate. Impairment in the formation of urea (in urea cycle) can occur either as a consequence of primary genetic defect or through secondary suppression of enzyme activities of urea cycle. Either of the process results in hyperammonemia. Sustained hyperammonemia in mice leads to increased lipid peroxidation in liver and brain, reflecting an oxidative stress condition.

α-ketoglutarate (α-KG) is an intermediate of citric acid cycle and is the natural ubiquitous collector of amino groups in body tissues. Further, an important function of α-KG occurs in the formation of carnitine. Because of its chemical structure, α-KG is a potent natural detoxifying agent. In some applications, it is far more powerful than vitamin C. One such application is as an antidote to cyanide poisoning where it binds to the cyanide and prevents the circulation of free cyanide. α-KG was found to offer protection in heart surgery and is known to decrease the levels of ischaemic markers, creatin kinase and troponin T. Conditions of hyperammonemia can be treated with α-ketoglutaric acid.

Systematic investigations on the levels of lipid peroxidation products and the levels of non-enzymatic and enzymatic antioxidants under the conditions of hyperammonemia are lacking. The present study deals with the systematic investigation on the levels of products of lipid peroxidation (thiobarbituric acid reactive substances—TBARS) and the levels of vitamins C and E (non-enzymatic antioxidants) and superoxide dismutase and catalase (enzymic antioxidants) under the conditions of hyperammonemia and during the treatment of α-KG in rats. Further, the levels of urea, non-protein nitrogen (NPN), serum transaminases and lipid profile variables (free fatty acids, triglycerides, phospholipids and cholesterol) have also been investigated.

Materials and Methods

Adult male Wistar rats (24; six months old) obtained from Central Animal House, Faculty of Medicine, Annamalai University were kept at room temperature (30°±2°C). Animals were randomized and
Chemical variables were calculated. Analysis of variance followed by 'Least Significant Difference' (LSD) was carried out to detect the significant differences between control and experimental groups.

Results

Body weight changes—Ammonium acetate treated rats (Group II) showed increases in body weight compared with controls (Table I). Ammonium acetate and α-KG treated rats (Group III and Group IV (α-KG treated rats) showed less significant differences in body weights when compared with control rats.

Biochemical analyses—The concentration of TBARS (Table 2) in plasma was increased significantly in ammonium acetate treated rats (Group II). Ammonium acetate and α-KG treated group showed significantly low levels of TBARS when compared with the corresponding ammonium acetate group (Table 2). α-KG treated rats (Group IV) showed significantly low levels of TBARS when compared to control rats. Similar patterns were observed on the levels of urea and non-protein nitrogen in between groups. Activities of liver marker enzymes, alanine transaminase (ALT) and aspartate transaminase (AST) were also altered in a similar manner in between groups.

administration of ammonium acetate caused a significant decrease in the levels of vitamins C and E in plasma when compared to controls. Ammonium acetate and α-KG treated groups showed significantly increased levels of vitamins C and E in plasma when compared with corresponding ammonium acetate treated group. α-KG treated rats showed significant increases in the levels of antioxidant vitamins (C and E) when compared with controls. Similar alterations were observed on the activities of enzymatic antioxidants (catalase and superoxide dismutase) in between groups.

Table 1—Body weight (g) changes in each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Ammonium acetate</th>
<th>Ammonium acetate + α-KG</th>
<th>α-KG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight</td>
<td>200.1 ± 4.8</td>
<td>205.0 ± 6.3</td>
<td>196.3 ± 3.5</td>
<td>199.0 ± 2.5</td>
</tr>
<tr>
<td>Final body weight</td>
<td>257.5 ± 2.7</td>
<td>295.8 ± 3.7</td>
<td>264.5 ± 8.8</td>
<td>263.8 ± 6.3</td>
</tr>
<tr>
<td>Net gain in weight</td>
<td>57.3 ± 4.2</td>
<td>89.1 ± 3.7</td>
<td>68.1 ± 6.7</td>
<td>64.8 ± 6.61</td>
</tr>
</tbody>
</table>

ANOVA followed by Least Significant Difference (LSD).

* Significant ($P<0.05$) when compared with control.

* Significant ($P<0.05$) when compared with ammonium acetate treated.
The levels of free fatty acids, triglycerides, phospholipids and cholesterol were significantly increased in ammonium acetate treated rats (Table 3). The levels were decreased in ammonium acetate and α-KG treated rats and were insignificantly different in α-KG treated group when compared with controls.

Discussion

The present results revealed that ammonium acetate treated rats gained significantly higher body weights compared to control rats. It may be due to increased levels of lipids during hyperammonemia as observed in the present study. Group III (α-KG with ammonium acetate) rats gained body weights similar to control rats. This could be due to detoxifying effects of α-KG.

It is a well established fact that ammonia intoxication enhances lipid peroxidation and generates free radicals. This could lead to increased levels of TBARS and decreased levels of enzymatic and non-enzymatic antioxidants in group II rats. Elevated levels of α-KG (in group III) could offer protection against oxidative damages by participating in the non-enzymatic oxidative decarboxylation in the hydrogen peroxide decomposition process.

Serum transaminases (ALT and AST) are sensitive indicators of liver cell injury. Elevated levels of ALT and AST in ammonium acetate treated rats might be related to damage and destruction of the liver tissue. By detoxifying ammonia, and also by elevating glutamate, glutamine, and other amino acids levels α-KG could be involved in wound repair of liver and could decrease the activities of transaminases.

Increased levels of urea and non-protein nitrogen may indicate the elevated levels of ammonia in ammonium acetate treated rats in the present study. α-KG collects amino (−NH₂) groups and traps ammonia.

Table 2 — Changes in the biochemical variables in four groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Ammonium acetate</th>
<th>Ammonium acetate + α-KG</th>
<th>α-KG</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (n moles/ml)</td>
<td>2.4±0.26</td>
<td>3.8±0.24</td>
<td>2.6±0.13</td>
<td>1.9±0.16</td>
<td>84.64</td>
</tr>
<tr>
<td>Vitamin C (mg/dl)</td>
<td>1.7±0.10</td>
<td>0.68±0.09</td>
<td>1.25±0.18</td>
<td>2.4±0.12</td>
<td>173.8</td>
</tr>
<tr>
<td>Vitamin E (mg/dl)</td>
<td>1.16±0.10</td>
<td>0.53±0.05</td>
<td>0.98±0.03</td>
<td>2.4±0.02</td>
<td>273.0</td>
</tr>
<tr>
<td>Catalase (µmoles/min/mgHb)</td>
<td>56.2±6.1</td>
<td>33.8±4.36</td>
<td>48.9±4.01</td>
<td>75.6±9.2</td>
<td>46.01</td>
</tr>
<tr>
<td>SOD (50% inhibition of NBT reaction/mgHb)</td>
<td>2.3±0.15</td>
<td>0.75±0.05</td>
<td>1.81±0.10</td>
<td>3.1±0.04</td>
<td>597.2</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>10.8±1.2</td>
<td>23.5±2.21</td>
<td>13.3±1.3</td>
<td>10.08±1.24</td>
<td>94.36</td>
</tr>
<tr>
<td>Non-protein nitrogen (mg/dl)</td>
<td>24.1±2.0</td>
<td>55.6±6.8</td>
<td>34.9±1.71</td>
<td>19.4±1.83</td>
<td>108.44</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>30.4±2.62</td>
<td>86.0±5.73</td>
<td>48.5±2.91</td>
<td>28.1±2.1</td>
<td>326.5</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>106.3±8.06</td>
<td>181.6±13.3</td>
<td>142.0±6.1</td>
<td>97.6±4.83</td>
<td>115.7</td>
</tr>
</tbody>
</table>

ANOVA followed by Least Significant Difference (LSD).

Table 3 — Changes in the lipid profile levels in four groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Ammonium acetate</th>
<th>Ammonium acetate + α-KG</th>
<th>α-KG</th>
<th>F-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fatty acids (mg/dl)</td>
<td>65.8±3.0</td>
<td>114.3±6.0</td>
<td>83.8±1.67</td>
<td>68.9±4.6</td>
<td>165.87</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>85.1±7.2</td>
<td>144.8±7.4</td>
<td>109.4±8.6</td>
<td>86.4±6.5</td>
<td>82.9</td>
</tr>
<tr>
<td>Phospholipids (mg/dl)</td>
<td>85.0±6.1</td>
<td>232.5±8.2</td>
<td>110.0±7.7</td>
<td>81.6±2.6</td>
<td>709.55</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>108.0±4.38</td>
<td>204.0±4.38</td>
<td>129.3±6.02</td>
<td>106.6±4.1</td>
<td>550.19</td>
</tr>
</tbody>
</table>

ANOVA followed by Least Significant Difference (LSD).

Table 2: Changes in the biochemical variables in four groups

Table 3: Changes in the lipid profile levels in four groups
in blood and in body tissues forming glutamate, glutamine and other amino acids by performing deamination and transamination reactions\(^5\) thus detoxifying ammonia.

Ammonium acetate could deplete the levels of $\text{a-KG}$ and other Krebs cycle intermediates\(^8\) and thus elevate the levels of acetyl CoA. The elevated levels of acetyl CoA could lead to increased levels of lipid profile (free fatty acids, triglycerides, phospholipids and cholesterol) as observed in the present study. Further, another important function of $\text{a-KG}$ occurs in the formation of carnitine\(^5,28\). Carnitine acts as a carrier of fatty acids into cell mitochondria so that proper metabolism of fats can proceed\(^29\). The decreased $\text{a-KG}$ levels in ammonium acetate treated rats could lead to accumulation of fatty acids which may be reversed during the treatment of $\text{a-KG}$.

In conclusion, exogenously administered $\text{a-KG}$ could cause the biochemical alterations by (i) detoxifying excess ammonia, (ii) participating in the non-enzymatic oxidative decarboxylation in the hydrogen peroxide decomposition process and (iii) enhancing the proper metabolism of fats.

References